(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
15 March 2001 (15.03.2001)

PCT

(10) International Publication Number
WO 01/17357 A1

(51) International Patent Classification[7]: A01N 47/44, 25/34, 25/24, 33/12

(21) International Application Number: PCT/US00/06053

(22) International Filing Date: 8 March 2000 (08.03.2000)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
09/392,842 9 September 1999 (09.09.1999) US

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(81) Designated States (*national*): AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, UZ, VN, YU, ZA, ZW.

(84) Designated States (*regional*): ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Published:
— *With international search report.*

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

WO 01/17357 A1

(54) Title: AMPHIPHILIC ANTIMICROBIAL FILM-FORMING COMPOSITIONS

(57) Abstract: The present invention relates to a topical antimicrobial composition containing an antimicrobial complex that provides sustained antimicrobial disinfecting action upon contact with microorganisms for prolonged periods, without the necessity for reapplication. The topical antimicrobial composition provides both initial and residual contact-killing disinfecting activity, and does not release its antimicrobial components into contacting liquids at levels that result in solution disinfection.

AMPHIPHILIC ANTIMICROBIAL FILM-FORMING COMPOSITIONS

Related Application Data

This application is a continuation-in-part of United States Serial No. 09/392,842, filed September 9, 1999, which claims priority to United States Serial No. 60/099,925, filed September 11, 1998, and to United States Serial No. 60/116,013, filed January 15, 1999. This application is also a continuation-in-part of United States Serial No. 09/248,861, filed February 11, 1999, which claims priority to United States Serial No. 60/074,456, filed February 12, 1998. The complete disclosures of each of the above documents are herein incorporated by reference.

Field of the Invention

The present invention relates to a composition that forms an adherent, transparent, water insoluble polymeric film on a substrate surface, and that provides sustained antimicrobial disinfecting action upon contact with microorganisms for prolonged periods, without the necessity for reapplication. The coating provides contact-killing surface disinfecting action only, and does not release its components into contacting liquids at levels that would result in solution disinfection.

Background of the Invention

The constant threat of bacterial contamination and the associated repercussions on health have made antimicrobial solutions a ubiquitous part of commercial and residential cleaning and disinfection processes. Dilute aqueous detergents show no detectable reduction in bacterial levels on surfaces amenable to bacterial growth and proliferation in susceptible environments, such as hospitals and in residential kitchen and bath areas. On the other hand, oxidants such as aqueous hypochlorite and phenolic compositions produce substantial reductions in bacterial levels that are relatively short-lived (3 to 6 hours). This often results in recontamination due to reuse of such surfaces, requiring frequent reapplication of disinfectant. Further, relatively high concentrations of the active agent have to be incorporated in such formulations to obtain broad-spectrum disinfection. These high concentrations often have undesirable side effects such as skin and eye irritation, in addition to being potentially hazardous when in contact with food. There is therefore a need for the development of new disinfecting formulations that can provide sustained broad spectrum microbial disinfection on surfaces over prolonged periods without reapplication,

even after being contacted by cleaning solutions and after surface reuse. Furthermore, it is desirable to achieve disinfecting action using low levels of the antimicrobial agent that will not pose toxicity problems for the user.

The modality of action of film-forming surface sanitizers to date has been solution based, that is, the antimicrobial action is obtained by controlled release via diffusion or dissolution of the active agents into contacting aqueous or volatile solutions. Numerous examples of this type of sanitizer have been reported. Another typical variant involves hydrolysis or dissolution of the matrix containing an antimicrobial compound, thereby effecting its release into solution. High levels of preservatives, however, are also released into contacting solutions in long-term applications. In such mechanisms, a bioactive compound is covalently bound either directly to the substrate surface or to a polymeric material that forms a nondissolving surface coating. The antimicrobial compounds in such coatings exhibit greatly diminished activity, unless assisted by hydrolytic breakdown of either the bound antimicrobial or the coating itself. In either case, relatively high levels of preservative have to be released into solution in order to elicit antimicrobial action.

Summary of the Invention

It is an object of the present invention to provide a non-eluting antimicrobial composition which is capable of (i) providing immediate broad-spectrum antimicrobial disinfection and (ii) providing sustained or residual antimicrobial disinfecting action for extended periods after application, even after being contacted by water or other liquids. An additional object of the invention is to provide a composition that can bind non-leachably to a surface. A further object of the invention is to provide an antimicrobial material that does not release biocidal amounts of leachables into a contacting solution. Another object of the invention is to provide a substantially water-insoluble, self-preserving microbial barrier film that imparts "persistence" (residual antimicrobial action) for extended periods after application. An additional object of the invention is to provide deodorizing action of extended duration on skin even after exposure to moisture and sweat. Another object of the invention is to provide an antimicrobial composition which comprises an optical reporter, e.g., a fluorophore or an optical brightening agent that enables detection of the presence of the antimicrobial composition on surfaces by suitable detection devices such as irradiation by an ultraviolet, fluorescent, infrared, or visible light source.

A further object of the present invention is to provide methods for detecting the presence of antimicrobial compositions on surfaces by providing an antimicrobial composition that contains an optical reporter. An additional object of the present invention is to provide methods for monitoring a subject's compliance with sterile or sanitary procedures by providing an antimicrobial composition that contains a marker and subsequently exposing the subject to a detector capable of detecting the presence of the marker on the subject in order to determine whether the subject applied the composition.

Another object of the present invention is to provide a non-eluting, self-preserving polymeric antimicrobial material that (i) forms a microbial barrier in-situ and (ii) is capable of inhibiting microbial growth and preventing microbes from growing through the barrier over extended periods. It is an object of the invention to render the self-preserving polymeric material substantially water-insoluble so as to make it non-eluting; that is, it does not dissolve, elute or leach into contacting aqueous solutions at bactericidal levels. This is accomplished by making it conducive to spontaneously associating with a surface and bonding to it via electrostatic, ionic or covalent bonding.

It is also an object of the invention to render the self-preserving polymer further water-insoluble by reacting it with a hydrophobic organic compound. Such a modification renders the polymeric antimicrobial material substantially water-insoluble, thereby enabling it to efficiently associate with a surface upon application.

Another object of the invention is to react the antimicrobial polymeric material with a covalent coupling agent so that the resulting adduct is capable of forming covalent chemical bonds with functionalities such as amino, sulfhydryl, or carboxylic acid groups. Such covalent bonding enables retention of the antimicrobial polymer upon its application to an appropriate surface. Thus, the antimicrobial polymer provides a non-leachable, non-eluting microbial barrier that is capable of rapid sanitation and persistent antimicrobial activity that is substantially undiminished even upon contacting water.

An additional object of the present invention is to provide an antimicrobial composition comprising an optional second antimicrobial component non-leachably dispersed in the self-preserving barrier-forming antimicrobial polymeric material such that the antimicrobial component is capable of enhancing the persistent antimicrobial efficacy of the composition by killing microorganisms on contact without leaching from the composition into the surrounding

environment at levels toxic to microorganisms. Such antimicrobial compositions are capable of providing residual antimicrobial activity on surfaces even after repeated exposure to aqueous solutions and thus are particularly useful as hard surface disinfectants and sanitizers, antifoulant coatings and topical dermal antiseptics.

The present invention provides a means of immobilizing a polycationic antimicrobial polymer on a substrate as a film. In some embodiments, the polycationic polymer is rendered substantially water-insoluble by the addition of a transition metal such as silver. In some embodiments, the solubility of the polymer is decreased by reacting the polymer with a hydrophobic compound. In some embodiments, the solubility of the polymer is decreased by mixing it with an anionic compound containing a hydrophobic moiety. These embodiments can be combined (*e.g.* by providing both the anionic compound and a transition metal), providing increased water resistance in some applications, or used separately (*e.g.* by providing the anionic compound in the absence of a metal) to still provide persistent antimicrobial activity, but in an embodiment that reduces production costs, facilitates manufacture, and/or facilitates removal of the film under defined conditions.

Thus, in some embodiments, the present invention relates to an antimicrobial composition comprising (i) an antimicrobial complex comprising an organic polycationic polymeric antimicrobial material and an antimicrobial metallic material wherein said metallic material is non-leachably bound to or associated with said organic polymeric antimicrobial material and (ii) a carrier, wherein the antimicrobial complex is dispersed within said carrier.

In these embodiments, the organic material should possess two important properties: it should be capable of reversibly binding or complexing with the biocidal metal, and should be capable of insinuating the biocidal metal into the cell membrane of a microorganism in contact with it. The organic material preferably is capable of disrupting or interacting with the cell membrane surrounding the microorganism. Preferred organic materials are those which can be applied on a surface as substantially water-insoluble films and which bind the biocide in such a manner as to permit transfer of the biocide into the microorganism without releasing the biocide (at biocidal levels) into the surrounding environment, e.g., into the air or into any liquid in contact with the coated surface. Preferred organic materials are polycationic polymeric antimicrobial materials such as biguanide polymers. Especially preferred biguanide polymers

include poly(hexamethylenebiguanide), poly(hexamethylenebiguanide) hydrochloride, or derivatives thereof.

In the embodiments of the invention that include a metallic material, the material is preferably an antimicrobial material that is toxic to microorganisms and is capable of complexing with or reversibly binding to the organic matrix material, thereby rendering the organic matrix substantially water-insoluble. The metallic biocide exhibits greater binding affinity to thiol functional groups in cellular proteins of microorganisms. When a microorganism contacts the polymeric organic material of the present invention, the polymer engages or disrupts at least the outer portion of the lipid bilayer of the microorganism's cell membrane sufficiently to permit insinuation of the metallic biocide into the microorganism, where cell proteins or proteins in the lipid bilayer compete effectively for the biocide due to favorable binding constants. Stated another way, the metallic material binds to or forms a complex with the organic material in which the association between the organic material and metallic material is sufficiently strong that the layer or film does not elute antimicrobial amounts of the metal into a contacting solution. However, the metallic material preferentially binds to thiol and amine functional groups in proteins in the microorganism and thus is transferred directly from the matrix to the microorganism. The antimicrobial metal is subsequently transported intracellularly and causes cell death. The result is a contact-killing delivery system that selectively transfers the metallic biocide to or into the microorganism's cell membrane upon contact, without elution or dissolution of the biocide into solution, thereby maintaining long term antimicrobial efficacy. Preferred metallic materials are silver or silver compounds and especially preferred compounds are silver iodide and silver nitrate.

Thus, the invention encompasses antimicrobial compositions in which a plurality of components cooperate to generate a coating that provides persistent antimicrobial activity that persists even after repeated contact with water. The antimicrobial materials of the present invention are, therefore, molecularly designed to enable a matrix-bound biocide to retain high antimicrobial activity without elution of any compounds into contacting solutions, carriers or other materials. The antimicrobial's activity stems from the sustained, cooperative biocidal action of its components. Selective transfer of one component from within the matrix directly to the microorganism upon contact may be achieved via a "handoff" mechanism upon engagement and penetration of the microorganism's cell membrane by the organic material. The

antimicrobial material, therefore, maintains long term efficacy without releasing toxic elutables into the surrounding environment. Components that can be used in the present invention to provide cooperative biocidal action can include both metallic and non metallic biocides.

The invention comprises compositions for immediate sanitation of a surface, providing long-term residual antimicrobial efficacy or over extended duration, even after being contacted with water under conditions simulating a hand rinse. In one embodiment, the formulation is a composition comprising a solution, dispersion or suspension of the organic polymeric antimicrobial material and the biocidal material in a suitable carrier. The composition need not be a homogeneous solution. If desired, stabilizing agents such as suspending agents or surface active agents may be included. The composition may also include an optical reporter, e.g., a fluorophore or an optical brightening agent that enables detection of the presence of the composition (by use of suitable detection devices, such as irradiation by an ultraviolet, fluorescent, infrared, or visible light source.

In another aspect, the invention relates to an antimicrobial film-forming composition containing a polycationic antimicrobial polymer and an anionic compound in a liquid carrier. The anionic compound includes a hydrophobic moiety and an anionic moiety. In one embodiment, the ratio of the number of anionic moieties (from the anionic compound) to the number of cationic sub-units (from the antimicrobial polymer) present in the carrier should be between 0.05 and 0.95. In another embodiment, the anionic moieties are phosphate or phosphonate groups and the ratio of the number of anionic moieties to the number of cationic subunits should be at least two, and preferably between two and about five. When present in a proper ratio, the anionic compound complexes with the polycationic polymer to deposit an amphiphilic, water-resistant antimicrobial film upon a contacting substrate. The film contains the anionic compound and the polycationic polymer and provides an antimicrobial activity that persists even after repeated contact with water. The hydrophobicity and hydrophilicity of this amphiphilic film can be modified by changing the ratio of the anionic and cationic components in the composition. Generally, compositions containing increasing amounts of the anionic compound are increasingly hydrophobic. The hydrophobicity may be further modified by reacting the polycationic polymer with a hydrophobic compound to form an adduct. Although the film is water-resistant, it can be removed by treatment with an alcohol such as ethanol. In other embodiments, a transition metal such as silver may be included to further reduce the water-

solubility of the film and provide an additional source of biocidal activity. Because the presence of the metal may also reduce the ethanol-solubility of the film and because of the expense of suitable metals such as silver, in many preferred embodiments the composition and the film do not contain silver or other transition metals, or do not contain them at levels sufficient to substantially reduce the ethanol-solubility of the film.

Accordingly, in one embodiment, the invention is an amphiphilic film-forming composition including a liquid carrier, an antimicrobial polymer with a plurality of cationic subunits, and a hydrophobic anionic compound with an anionic moiety and a hydrophobic moiety, wherein the mole ratio of anionic moieties to cationic subunits is between 0.05 and 0.95. The polymer is preferably a biguanide polymer of at least three subunits, and may be a hydrophobic adduct thereof. The anionic moiety is preferably a sulfate, a sulfonate, or a carboxylate; the hydrophobic adduct preferably includes an aliphatic moiety of at least twelve carbon atoms. The carrier may include water, a polar organic solvent, or mixtures thereof. The composition may or may not include a metal such as silver.

In another embodiment, the invention is an antimicrobial film including an antimicrobial polymer with a plurality of cationic subunits and a hydrophobic anionic compound with a hydrophobic moiety and an anionic moiety, wherein the mole ratio of anionic moieties and cationic subunits is between 0.05 and 0.95. The film is resistant to extraction with water and provides a persistent antimicrobial activity against contacting microorganisms.

In another embodiment, the invention is an amphiphilic film-forming composition including a liquid carrier, an antimicrobial polymer with a plurality of cationic subunits, and a hydrophobic anionic compound with a hydrophobic moiety and a phosphate or a phosphonate moiety, wherein the mole ratio of phosphate or phosphonate moieties to cationic subunits is at least two.

In a related aspect, the invention relates to a method of preparing a film-forming composition. An antimicrobial polymer is dissolved or transferred to a polar organic solvent to which is added a solution including an anionic compound with a hydrophobic moiety and an anionic moiety. Preferably, the addition is gradual with mixing to minimize the risk of premature precipitation. In one embodiment, the final ratio of the number of anionic moieties (from the anionic compound) to the number of cationic moieties (from the polymer) should be between 0.05 and 0.95. In this embodiment, the anionic moieties are preferably sulfates,

sulfonates, or carboxylates. In another embodiment, the anionic moieties are phosphates or phosphonates and the final ratio is at least two. If the resulting composition is contacted with a substrate, the composition deposits on the substrate a film that provides a persistent, water-resistant antimicrobial activity.

Accordingly, in one embodiment, the invention is a method of preparing a film-forming composition by providing, in a polar organic solvent, an antimicrobial polymer with a plurality of cationic subunits, and adding to the solution an anionic compound with a hydrophobic moiety and an anionic moiety. The final molar ratio of the anionic moieties and the cationic subunits should be between 0.05 and 0.95.

In another embodiment, the invention is a method of immobilizing an antimicrobial polymer on a substrate by preparing, in a polar organic solvent, an anionic compound with a an anionic moiety and a hydrophobic moiety and an antimicrobial polymer with a plurality of cationic subunits. The concentration of the polar organic is always at least 0.5% (w/v) and the mole ratio of anionic moieties and cationic subunits is never more than five. The final mole ration of anionic moieties and cationic subunits is between 0.05 and 0.95. A substrate is contacted with the resulting composition, such that the antimicrobial polymer and the anionic compound form a water-resistant film on the substrate.

The present invention discloses a method to enhance the activity of antiseptic formulations that overcome the limitations of formulations known in the prior art and can be used in all of the above-mentioned categories of antiseptics and disinfectants, since they confer on such formulations antimicrobial persistence that remains unaffected even after loss of active ingredients in the antiseptic by evaporation or dissolution by water contact. The antimicrobial materials of the present invention may be used to enhance the efficacy of commercial biocidal compositions and enable such formulations to exhibit persistent antimicrobial efficacy even upon being contacted with water. Furthermore, the compositions of the present invention provide such antimicrobial activity without producing skin irritation or cytotoxicity due to their non-eluting character.

The limitations in the prior known methods and compositions are overcome by the present invention which relates to the addition of a self-preserving film-forming polymeric antimicrobial agent that enhances the efficacy of alcohol-containing formulations and provides residual antimicrobial efficacy or persistence after alcohol evaporation, thereby allowing more

efficient use of alcohols as disinfectants or antiseptics. Additionally, the present invention relates to the in-situ formation of a microbial barrier or film. This microbial barrier or film is self-preserving; it kills contacting microorganisms and prevents them from growing through or penetrating the barrier.

In another aspect, the present invention relates to compositions comprising an antiseptic formulation and an organic, polycationic, antimicrobial polymer that binds to a surface upon application. In one embodiment, the formulation spontaneously binds to a surface upon application, forming a self-preserving antimicrobial barrier that provides persistent antimicrobial activity.

These and other objects, features and advantages of the present invention will be better understood from the following description when read in conjunction with the accompanying drawings and examples.

Brief Description of the Drawings

Figure 1A is a schematic graphic illustration of the polymer/biocide complex of the present invention, forming a film on the surface;

Figure 1B is a schematic graphic illustration of the contact-killing ability of the film-forming matrix/biocide complex of the present invention upon contact of the film with microorganisms, wherein the polymer chains engage and disrupt the microorganism cell membrane; and

Figure 1C is a schematic graphic illustration of the penetration of the cell membrane and transfer of the biocide from the network to proteins in the microorganism, causing cell death.

Figure 2 is a graph of the percentage of PHMB or M-PHMB from various antimicrobial coatings retained on the substrate surface after a number of 1-hour extraction cycles using hard water (50 ppm $CaCO_3$) at 50°C. The x-axis represents the number of one-hour extraction cycles, whereas the y-axis represents the percent of PHMB or M-PHMB retained on the substrate surface.

Figure 3 is a graph of the residual efficacy of various antimicrobial coatings on ceramic tile after repeat challenges with *P. aeruginosa* (PSA) shown as a function of Log_{10} reduction from a control versus the number of challenges. The x-axis represents the number of challenges; the y-axis represents the log_{10} reduction in viability when compared to the control.

Detailed Description

The antimicrobial compositions of the present invention can be applied directly to a surface to disinfect the area of application upon contact. The antimicrobial compositions also provide residual activity to kill microorganisms contacting the area of application subsequent to the initial treatment.

The term "microorganism" as used herein includes pathogenic organisms and infective agents, including bacteria, viruses, blue-green algae, fungi, yeast, mycoplasmids, protozoa, parasites and algae.

The term "biocidal" as used herein means bactericidal or bacteriostatic. The term "bactericidal" as used herein means the killing of microorganisms. The term "bacteriostatic" as used herein means inhibiting the growth of microorganisms, which can be reversible under certain conditions.

As used herein, the terms "non-eluting", "non-leachable" and "substantially non-leachable" mean that bioactive components in the disinfecting compositions do not dissolve, elute, leach or otherwise provide species into a liquid environment in contact with the compositions at levels that would result in solution disinfection, that is, in antimicrobially effective amounts. Preferably, this threshold is below the minimum solution inhibitory concentrations (MIC) of such components to cause the contacting solution to be biocidal.

As used herein, the terms, "sanitizer" and disinfectant refer to those that are applied to hard surfaces for the purposes of killing bacteria and microorganisms on the surfaces. "Antiseptic" refers to those mixtures that are applied to skin for the purpose of killing bacteria and microorganisms on the skin. Other uses will become apparent to those skilled in the art and are intended to be within the scope of this invention.

The phrase "self-preserving antimicrobial barrier or film" as used herein refers to any antimicrobial polymeric compound that is capable of forming a barrier or film on the surface of a substrate, and inhibits the proliferation of microorganisms on said film, and prevents them from growing through to the underlying surface. The phrase "residual antimicrobial activity" as used herein refers to the activity of any chemical compound that is capable of forming a residue on a substrate surface and, upon its application, is capable of providing either bacteriostatic or bactericidal activity. When present in an antiseptic or disinfectant formulation containing other active antimicrobial agents, the residue obtained from such agent is capable of sanitizing (bactericidal action) or acting as a preservative to prevent organism growth (bacteriostatic

action). The term "residual efficacy" as used herein refers to the ability of an antimicrobial material to kill bacteria and microorganisms for an extended period of time after the initial disinfecting or sanitizing action caused by application of the antimicrobial formulation. The term "persistence" as used herein refer to the ability of an antimicrobial material to inhibit bacterial regrowth for an extended period of time after the initial antiseptic action caused by application of the antimicrobial formulation.

As used herein, "cationic subunit" denotes a subunit that normally bears one or more positive charges when in aqueous solution at a neutral pH. A cationic subunit preferably bears a net positive charge of about +1 or more when in aqueous solution at a neutral pH.

As used herein, "anionic moiety" denotes a chemical moiety that normally bears one or more negative charges when in aqueous solution at a neutral pH.

As used herein, "polyoxoanion moiety" denotes an anionic moiety that includes at least two oxygen atoms. Examples of polyoxoanion moieties include sulfates, sulfonates, phosphates, phosphonates, borates, and carboxylates.

As used herein, "anionic compound" denotes a compound that includes an anionic moiety and a hydrophobic moiety. The anionic compound preferably bears a net negative charge of about 1 or more (*e.g.* a net charge of about –2, about –3, *etc.*) when in aqueous solution at a neutral pH.

Organic materials useful in the present invention comprise antimicrobial materials that are capable of: (1) adhering to and/or forming a layer or coating on a surface such as skin, (2) reversibly binding to or complexing with a biocide to prevent its elution or dissolution, and (3) insinuating the biocide into the cell membrane of contacting microorganisms. A preferred class of materials is the class having the aforementioned properties, which are capable of being immobilized on a surface and which preferentially bind to biocidal materials (especially metallic biocides) in such a manner so as to permit release of the biocide to the microorganism, but not to the contacting environment. Most preferred is the class of organic materials having antimicrobial properties: materials that, when applied as a coating, can dissolve into, adhere to, disrupt or penetrate the lipid bilayer membrane of a microorganism in contact with the barrier film. In a preferred embodiment, the organic material is a polymer containing segments which, when the polymer forms a coating on a surface, are capable of engaging microorganisms which come into

contact with the coating. By "engaging" it is meant that the coating can attach and temporarily immobilize a microorganism in contact with it. The barrier film can dissolve into, or adhere to, and penetrate at least the outer portion of the lipid bilayer membrane of a microorganism. For this purpose, surface active agents, such as cationic compounds, polycationic compounds, anionic compounds, polyanionic compounds, non-ionic compounds, polyanionic compounds or zwitterionic compounds may be used. These compounds include, for example, biguanide polymers, or polymers having side chains containing biguanide moieties or other cationic functional groups, such as benzalkonium groups or quarternium groups (e.g., quarternary amine groups). The polymer backbone may be any polymer capable of forming a coating on a substrate. It is understood that the term "polymer" as used herein includes any organic material comprising three or more repeating units, and includes oligomers, polymers, copolymers, terpolymers, etc. The polymer backbone may be a polysilane or polyethylene polymer, for example. Organic materials which currently are most preferred for use in the invention are polymeric biguanide compounds. When applied to a substrate, these polymers form a barrier film that can engage and disrupt a microorganism as shown in Figure 1.

Polymeric materials useful in the present invention include polymers containing benzalkoniumchloride or its derivatives, α-4-[1-tris(2-hydroxyethyl) ammonium-2-butenyl] poly[l-dimethylammonium-2-butenyl]-ω -tris(2-hydroxyethyl) ammonium chloride. Preferred polymeric compounds include polymeric biguanides and their salts of the general formula:

$$
\begin{array}{c}
Y_1\text{-}[\text{-NH-}\underset{\substack{\parallel \\ NH^+ \\ Z^-}}{C}\text{-NH-}\underset{\substack{\parallel \\ NH^+ \\ Z^-}}{C}\text{-NH-X-}]_n\text{-}Y_2
\end{array}
$$

or their water soluble salts, where X is any aliphatic, cycloaliphatic, aromatic, substituted aliphatic, substituted aromatic, heteroaliphatic, heterocyclic, or heteroaromatic compound, or a mixture of any of these, and Y_1 and Y_2 are any aliphatic, cycloaliphatic, aromatic, substituted aliphatic, substituted aromatic, heteroaliphatic, heterocyclic, or heteroaromatic compound, or a mixture of any of these, where n is an integer equal to or greater than 1, and wherein Z is an anion such as Cl^- or OH^-. In a preferred embodiment, the polymeric material is capable of adsorbing to a surface via electrostatic interaction, ionic interaction, or "hydrophobic forces". In another preferred embodiment, the polymeric material can bond covalently with a surface. Currently, the most preferred polymeric compound is polyhexamethylenebiguanide hydrochloride (available from Avecia, Inc. of Wilmington, DE as a 20% aqueous solution under

the trade names VANTOCIL™ and COSMOCIL-CQ™). Similarly preferred polymeric compounds include poly(hexamethylenebiguanide) hydrochloride, poly(hexamethylenebiguanide) gluconate, or poly(hexamethylenebiguanide) derivatives.

In another preferred embodiment, the organic polymeric material may be further reacted with a substantially water-insoluble organic compound or "hydrophobic agent" to form a substantially water-insoluble adduct that is capable of forming a amphiphilic barrier or film *in situ* that is impervious to contact with water or aqueous solutions. As used herein, "substantially water-insoluble" means that bioactive components in the disinfecting compositions do not dissolve, elute, leach or otherwise provide species into a liquid environment in contact with the compositions at levels that would result in solution disinfection, that is, in antimicrobially effective amounts. Preferably, this threshold is below the minimum solution inhibitory concentrations (MIC) of such components to cause the contacting solution to be biocidal. This adduct, when added to an antiseptic formulation or other carrier, confers on it a residual antimicrobial activity or persistence for extended periods of time in water-contacting (aqueous) environments. In a preferred embodiment, the organic material is a polymeric polycationic polymer, which is chemically reacted with a hydrophobic agent to form an adduct. The adduct that includes the hydrophobic agent exhibits greater water-insolubility, thus adhering more strongly to a surface than does the polycationic polymer alone. Hydrophobic agents which can be used in the present invention are organic compounds which are substantially water-insoluble and which can react with the polycationic material to form an adduct. Suitable hydrophobic agents include, for example, organic compounds containing a multifunctional groups such as a carbodiimide, isocyanate, isothiocyanate, succimidyl ester, epoxide, carboxylic acid, acid chloride, acid halide, acid anhydride, succimidyl ether, aldehyde, ketone, alkyl methane sulfonate, alkyl trifluoromethane sulfonate, alkyl paratoluene methanesulfonate, alkyl halide and organic multifunctional epoxide. In a currently preferred embodiment, a polyhexamethylene biguanide polymer is reacted with an epoxide, such as methylene-bis-N,N-diglycidylaniline, bisphenol-A-epichlorohydrin, or N,N-diglycidyl-4-glycidyloxyaniline. The degree of hydrophobicity of the resulting adduct can be adjusted by choice of the hydrophobic agent. The organic material can be polymeric or non-polymeric, and the resulting adduct may be capable of forming a coherent film.

In embodiments in which the film is non-permanent, covalent bonding of the film to a substrate or chemical crosslinking of the film is normally undesirable, since it would preclude their removal from the substrate with alcoholic or surfactant solutions. In these embodiments, the adduct can be treated to prevent any remaining reactive groups in the adduct from later forming additional covalent bonds (*e.g.* with a surface) or participate in chemical crosslinking reactions. For example, where the adduct contains or is formed by reaction of epoxides, the adduct is generally formed at an alkaline pH to prevent acidic hydrolysis of the epoxide residues. The resulting adduct can be subsequently treated with an acidic solution (*e.g.* 2N HCl in ethanol) to hydrolyze any remaining epoxide residues. The absence of epoxide residues can then be confirmed by infrared spectroscopy, chemical analysis, or other methods known in the art.

In another embodiment, the polycationic polymer is combined with an anionic compound containing a hydrophobic moiety. The resulting composition has a reduced solubility in water, but remains soluble in a polar organic solvent such as ethanol or isopropyl alcohol, for example. Without wishing to be bound by any theory, it is envisioned that the anionic group or groups in the anionic compound interact with the cationic subunits of the polymer, exposing the hydrophobic moieties to the external solvent. When the composition is contacted by a surface, the composition is deposited as a film on the surface. It is envisioned that, within such a film, the polycationic polymer would contact the surface and be immobilized by any of a variety of chemical forces including ionic forces, van der Waals forces, and "hydrophobic forces". Similarly, it is envisioned that the hydrophobic moieties may be, at least in part, on the external portion of such a film. Whether or not this is the film's true chemical structure, the film is water-resistant and provides an antimicrobial activity that persists even after repeated contact with water. Furthermore, the biocidal components of the film do not leach from it into contacting water at biocidal levels. Because the film contains both hydrophilic and hydrophobic components, the film can be deposited on a substrate whose surface is hydrophilic, hydrophobic, or amphiphilic.

The anionic group or groups in the anionic compound may include, but are not limited to, polyoxoanions such as sulfate, sulfonate, phosphate, phosphonate, borate, and carboxylate. The anionic group may include other hetero atoms such as thiocarboxylate. The hydrophobic moiety is preferably an aliphatic moiety that is preferably unbranched and unsubstituted. The hydrophobic moiety preferably contains at least twelve carbon atoms. More preferably, the

hydrophobic moiety contains an aliphatic substituent containing at least twelve carbon atoms, optionally as part of an aliphatically-substituted aryl moiety. Alternatively, the hydrophobic moiety may be a silicon-containing compound such as a silicate or a siloxane. Particularly preferred anionic compounds include dodecyl sulfate, stearate, and dodecylbenzenesulfonate.

In these embodiments, the relative ratios of the anionic compound to the polycationic polymer are very important. If too little of the anionic compound is present (below a critical ratio), the composition will not be substantially water-insoluble. If too much is present (above optimal ratio), the solution may become unstable and gel or form precipitates. The critical and optimal ratios in the embodiments are dependent on the nature of the polyoxoanion species. If the concentration of the anionic compound exceeds its critical micelle concentration (CMC), the resulting composition will not deposit a water-resistant film on a contacting substrate. Excessive levels of the anionic compound may also interfere with chemical interactions between the polycationic polymer and the substrate, such that even if a film is deposited, it may not stably interact with the substrate, increasing the risk of delamination. Thus, for anionic compounds containing a sulfate moiety, a sulfonate moiety, or a carboxylate moiety, the ratio of the number of anionic moieties to the number of cationic subunits should be between 0.05 and 0.95. More preferably, the ratio should be between 0.2 and 0.8. Ideally, the ratio should be about 0.5. In contrast, for anionic compounds containing phosphate or phosphonate, the ratio should be at least two, and preferably from about two to about five.

Because these compositions have reduced solubility in water, they are preferably generated initially in a liquid carrier including one or more polar organic solvents. Suitable solvents include, but are not limited to, C_1 to C_4 alkanols, C_1 to C_4 nitriles, ketones with three to eight carbon atoms, dimethylsulfoxide, and N,N-dimethylformamide. Ethanol is a preferred solvent. The polar organic solvents may be mixed with each other, with other organic solvents, and/or with water. Although it is preferable to formulate the composition initially in a polar organic solvent, the composition may be present entirely in water as a colloidal suspension, for example, similar to a latex paint. In such an embodiment, the liquid carrier preferably comprises at least twenty percent water. The carrier may optionally include one or more surfactants (*e.g.* nonionic detergents) to further stabilize a colloidal suspension, for example. The composition is preferably maintained between pH 4 and pH 10, and more preferably, between pH 6 and pH 8.

Thus, in one preferred embodiment, the antimicrobial composition binds non-leachably to a surface by virtue of hydrophobic-hydrophobic interactions. In another embodiment, the antimicrobial composition binds non-leachably to a surface by virtue of electrostatic interactions. In another embodiment, the antimicrobial composition binds non-leachably by virtue of one or more covalent bonds formed between the composition and the surface. These embodiments are not exclusive: the binding of the antimicrobial composition to the surface could be stabilized by any combination of the above elements. In a preferred embodiment, one or more of these interactions occurs rapidly following application of the embodiment on a surface. In a particularly preferred embodiment, these interactions can form at approximately room temperature, *i.e.* at about twenty to twenty-five degrees Celsius.

In some embodiments, the polymeric antimicrobial material is preferably formulated in a composition that includes a second biocidal agent. This second agent comprises any antimicrobial material that is capable of non-leachably binding to, interacting with or complexing with the polymeric material, but which, when placed in contact with the microorganism, preferentially transfers to the microorganism. For this purpose, antimicrobial metallic materials which bind to cellular proteins of microorganisms and which are toxic to microorganisms are preferred. The metallic material can be a metal, metal salt, metal complex, metal alloy or mixture thereof. Metallic materials that are bactericidal or bacteriostatic and are substantially water-insoluble or can be rendered substantially water-insoluble are preferred. By a metallic material that is bacteriostatic or bactericidal is meant a metallic material that is bacteriostatic to a microorganism, or that is bactericidal to a microorganism, or that is bactericidal to certain microorganisms and bacteriostatic to other microorganisms. Examples of such metals include, e.g., silver, zinc, cadmium, lead, mercury, antimony, gold, aluminum, copper, platinum and palladium, their oxides, salts, complexes and alloys, and mixtures of these. The appropriate metallic material is chosen based upon the ultimate use of the composition. The currently preferred metallic materials are silver compounds. In a currently preferred embodiment, a silver halide is used, most preferably, silver iodide. In another preferred embodiment silver nitrate is used which is converted into a substantially water-insoluble silver halide by subsequent chemical reaction with an alkali halide. Most preferably, silver nitrate is converted in-situ to silver iodide by reacting it with sodium or potassium iodide.

The compositions of the present invention are prepared by mixing, dispersing or blending the antimicrobial complexes with the carrier. It is preferable, but not necessary, to thoroughly blend the antimicrobial complex with the carrier to form a homogeneous mixture. The final concentration of the antimicrobial complex in the composition may vary depending on the intended use and particular formulation of the ultimate composition, as will be appreciated by those of ordinary skill in the art. The final concentration of the organic polycationic material may range from 0.5% to 50% by weight. Concentrations of the metallic material, if present, may range from 0.05% to 5% by weight.

Embodiments of the present invention including a polycationic polymer and a hydrophobic anionic compound are preferably formulated in a polar organic solvent. Preferably, the polycationic polymer is first prepared as an aqueous solution that is then mixed with a polar organic solvent. To this solution the hydrophobic organic compound (preferably in aqueous solution) should be mixed slowly, with stirring. If, instead, the solution containing the polymer is added to the solution containing the hydrophobic organic compound, the composition may become unstable, leading to formation of a gel or a precipitate.

The antimicrobial compositions of the present invention can also include an optical reporter, e.g., a fluorophore or an optical brightening agent that enables detection of the presence of the antimicrobial composition by use of suitable detection devices, such as irradiation by an ultraviolet, fluorescent, infrared, or visible light source. A preferred optical reporter is fluorescent brightener 28 (UVTex-OB, Ciba Specialty Chemicals Corp., Tarrytown, NY). Another preferred optical reporter is Tinopal SFP. When a treated surface is examined under light (UV radiation at 365 nm), these optical reporters fluoresce, thereby confirming the presence of the antimicrobial composition. The optical reporter may be blended into the antimicrobial composition to a final concentration in the range of 0.05% to 5% by weight, and most preferably around 0.15% by weight.

Methods of the present invention relate to detection of the presence of the antimicrobial composition by first providing the antimicrobial composition plus optical reporter as described above, then exposing the surface of interest to a detector capable of detecting the presence of the optical reporter. These methods can be utilized as part of a coherent sanitary program to monitor compliance with sterile procedures in healthcare environments and food establishments.using methods that comprise treating a surface with a antimicrobial composition including an optical

reporter of the present invention, and subsequently exposing the surface to a detector capable of detecting the presence of the optical reporter on the surface.

The invention is further illustrated by the following examples, which are not intended to be limiting in any way.

Example 1

Coating Application

The antimicrobial films can be applied by spraying over the surface of ceramic tiles (using a standard 22-oz trigger bottle) and spreading the liquid evenly with a soft tissue. One square foot of a tile surface is treated at once (64 tiles, each 35mm x 35 mm in size). Alcohol based formulations are dried for 10 minutes, and aqueous or DMF based formulations are dried for 30 minutes.

Antimicrobial activity testing

The coated samples are tested for antimicrobial efficacy in the following tests:

A. Standard bactericidal test for hospital grade disinfectant;

B. Standard fungicidal test for hospital grade disinfectant;

C. Standard virucidal test for hospital grade disinfectant;

D. Residual efficacy test for disinfectant;

E. Multiple microbial challenge test for sustained residual disinfection;

F. Antimicrobial efficacy of the coating after one exposure to common bathroom cleaning solutions;

G. Coating antimicrobial efficacy in the presence of growth supporting media; and

H. Kirby-Bauer zone of inhibition test, which should not show a zone of inhibition, indicating that the coatings are non-leachable.

A-C. Standard tests for bacterial, viral and fungicidal activity

Tests are run per American Society of Analytical Chemists (AOAC) protocols.

Formulation is applied to glass slides subjected to bacterial, fungal and viral challenge per standard test conditions. The formulation is disinfecting, virucidal and fungicidal under these test conditions.

D. Sustained residual disinfection testing

Tests are done on residual film on glass slides and ceramic tiles after evaporation of solvents.

The residue is immersed in water at 25°C for 24 hours, after which it is challenged with *Psedomonas aeruginosa, Staphylococcus aureus and Salmonella choleraseius.* Tiles and slides containing residue after water contact are inoculated with 0.3 ml of a microorganism suspension in PBS. Incubation of microorganisms is carried out at ambient temperature (20°C) in a humidity chamber. Planctonic microorganisms are recovered from the tile surface, then serial dilutions are performed and plate counted by standard techniques.

E. ATT coating performance in multiple microbial challenge test

Nonporous glazed tiles, 35x35 mm size, treated with a film-forming composition is tested for antimicrobial efficacy in a multiple challenge test with gram negative bacteria *Pseudomonas aeruginisa* and *Escherichia coli*; gram positive bacteria, *Staphylococcus aureus*; fungus *Aspergillus niger* (mold); and *Candida albicans* (yeast).

Tiles are repetitively inoculated with 0.3 ml of a microorganism suspension in PBS. Between the inoculations the tile samples are rinsed with tap water (250 ml per tile) and air-dried. No sterilization of the samples is performed between inoculation cycles. Incubation of microorganisms is carried out at ambient temperature (20°C) in a humidity chamber. Planctonic microorganisms are recovered from the tile surface, then serial dilutions were performed and plate counted by standard techniques.

<u>Example 2</u>

Contact Test for Biocidal Activity

Ceramic tiles are spray-coated with a film-forming composition according to the procedure described in Example 1.

The surface biocidal activity is tested according to the following procedure:

Cultures are prepared of the following microorganisms:

Escherichia coli (ATCC #8739)

Pseudomonas aeruginosa (ATCC #9027)

Salmonella cholerasius (ATCC#10708)

Staphylococcus aureus (ATCC #6538)

Candida albicans (ATCC #9642)

Aspergillus niger (ATCC #9027)

Inoculi are prepared from cultures of these microorganisms according to known procedures. To test the antimicrobial efficacy of the present coatings, ceramic tiles treated with the coatings and untreated control tiles are sprayed with 10^6 cfu/ml (cfu = colony forming units) of each of the above organisms. The tiles are incubated for 20 hours in a 25°C humidity chamber. The number of viable organisms on the surface are then determined by swabbing the surfaces of the tiles and culturing the organisms collected from the surface by the spread plate method. The presence of the two fungus samples (*Candida* and *A. niger*) also are determined by the turbidity method on PBS extracted swabs.

Example 3

Efficacy After Exposure to Water

The ceramic tiles from Example 2 are further tested to determine antimicrobial efficacy after washing. To simulate long-term use, the tiles are washed with tap water for 2 hours. (total volume, 1 gal/in^2). The biocidal and antifungal challenge tests described in Example 2 are repeated.

Example 4

Kinetics of Bactericidal Activity

Ceramic tiles are sprayed with film-forming compositions according to the procedures described in Example 1. Some of the sprayed tiles and unsprayed controls are placed under running tap water for 2 hours (total volume, 1 gal/in^2) of the following organisms: *P. aeruginosa, S. aureus* and *E. coli*. The tiles are incubated at ambient temperatures, and the number of viable organisms was determined quantifiably by the spread plate method at different time points. The pre- and post-washed spray antimicrobial efficacies are determined as a function of time.

Example 5

Efficacy After Repetitive Challenge

Treated ceramic tiles and untreated control tiles are challenged with 10^5 cfu/ml *P. aeruginosa.* The tiles are incubated at 30°C for 3 hours or for 20 hours in a humidity chamber, after which the presence of viable organisms is determined by the spread plate method. The tiles are rinsed with water (250 ml/tile) and the bacterial challenge is repeated. This cycle is repeated to obtain a total of 10 challenges each for the 3 hour and 20 hour incubations.

Example 6:

Preparation of Coating Composition

10 g of a 20% aqueous solution of polyhexamethylene biguanide hydrochloride (PHMB.HCl) was added, with stirring, to a solution containing 688 g of ethanol and 290 g of water. To this a solution of 1.3g of sodium dodecylsulfate dissolved in 10g of water was added slowly while stirring to give a stable clear solution.

Example 7:

Deposition of water resistant antimicrobial films from solutions of polymeric biguanide and polyoxo ligands

Polyhexamethylenebiguanide (PHMB) and compounds comprising polyoxo ligands were prepared by anion exchange reaction between PHMB hydrochloride (PHMB*HCl) and sodium salts of the ligand compounds in aqueous ethanol using the method described in Example 6. In all experiments the concentration of PHMB*HCl was maintained at 0.2% (wt./v). The molar ratio between PHMB*HCl and the acid varied in a range from 10 to 0.2. Ethanol content in final formulations was approximately 70% by volume.

The formulations were applied onto the surface of glass slides at a ratio 50 uL per square inch and air dried for 1 hour. Slides were exposed to DI water at the ratio of 10 ml per cm^2 for 1 hour at 40°C. After water contact the glass slides were quickly rinsed with DI water and stained with Eosin Y (at room temperature for 5 minutes) to estimate the amount of PHMB left on the glass surface after contact with water. The intensity of staining was evaluated by a color intensity chart and given a relative score from 0 to 10. The results shown in Table I below.

Table I:

Surfactant	Coating staining intensity at given surfactant/PHMB molar ratio, relative score (0 to 10)					
	0	0.1	0.5	1.0	2.0	5.0
2-Acrylamido-2-methyl-1-propanesulfonic acid	4 -	4-	4-	5-	5-	4-
Dodecylbenzenesulfonic acid, sodium salt	4 -	7+	8+++	7++	7++	5+
Stearic acid, sodium salt	4 -	9+	9++	10+++	9+++	4 ++
Ethylenediaminetetraacetic acid, tetrasodium	4 -	5 -	6-	5-	5-	6-
Sodium dodecyl sulfate	4 -	7+	10+++	9++	8+	5-
Sodium xylenesulfonate	4 -	5-	5-	5-	5-	5-
Sodium borate	4 -	4 -	4 -	4 -	4 -	4 -
Sodium methane sulfonate	4 -	4 -	4 -	4 -	4 -	4 -
Dodecyl phosphate, disodium**	4-	4-	4-	5+	7++	9++
Octadecyl phosphonate, disodium***	4-	4-	5 -	6 -	6 -	8 -/+

*) "+" sign indicates amount of residue visually observed on the surface of the slide, "-" sign indicates absence of residue or presence of the film.

**) Solutions containing reagents at 1:2 and 1:5 ratio not stable and contain substantial amount of gel-type precipitate.

***) Solutions are not stable at any given reagent ratio, films deposited initially were completely removed by water (with exception of the sample with PHMB/phosphonate molar ratio 0.2, where approximately 30% of the surface was covered with loosely bound PHMB containing particles).

From these results, it can be seen that carboxylic acids, sulfates, sulfonates, phosphates, and phosphonates containing aliphatic substituents (preferably C_{12} and higher) or aliphatically substituted (preferably C_{12} and higher) aryl radicals form coating solutions in which the water solubility of PHMB is substantially lowered. Such solutions obtained in situ as the result of the ion-exchange reaction between PHMB hydrochloride and sodium salt of corresponding acid can be deposited as a continuous film on the surface of solid non-porous substrates. The deposition of these films can be accomplished from aqueous solutions containing a water miscible organic

solvent (ethanol, acetone, acetonitrile, etc.) or other dispersion aid. Coatings, once deposited, are hydrophobic, are resistant to removal by water and remain antimicrobially active.

Example 8:

Water resistance of insoluble PHMB-polyoxo ligand antimicrobial coating in water

The salts of polymeric biguanides (PHMB) and dodecylsulfuric (DS) acid were prepared by anion exchange reaction between PHMB hydrochloride and sodium dodecylsulfate in aqueous ethanol (EtOH) solution, as discussed in Examples 6 and 7.

PHMB containing products used in this experiment were polyhexamethylenebiguanide hydrochloride (PHMB*HCl) and copolymer of polyhexamethylenebiguanide hydrochloride with methylenebisdiglycedylaniline (M-PHMB*HCl). The molar ratio between PHMB and DS was 1:0.5 for both polymeric salts prepared. The PHMB-DS compounds obtained as well as initial PHMB hydrochlorides were diluted with 70%(wt.) aqueous EtOH to get final concentration of PHMB base units of 0.165% for PHMB*HCl based solutions, and of 0.120% for M-PHMB*HCl based solutions.

The formulations were applied onto the surface of glass slides at a ratio 100 uL per sample ($20 cm^2$) and air dried for 1 hour. Initial PHMB surface content was 7.1 $\mu g/cm^2$ for PHMB*HCl based coatings and 5.2 $\mu g/cm^2$ for PHMB*HCl-MBDGA based coatings.

Coated glass slides were contacted with water in several consecutive extraction cycles. Water with controlled hardness (50 ppm $CaCO_3$) was used for extraction at a volume of 50 ml per glass slide. Results are shown in Figure 2 and below in Table II.

Table II. PHMB remaining on the surface after each extraction cycle as a % of initial amount applied on surface

Extraction cycle, hr	PHMB-HCl	PHMB-DS	M-PHMB	M-PHMB-DS
0	100.0%	100.0%	100.0%	100.0%
1	28.0%	61.6%	26.3%	77.0%
2	19.4%	54.3%	24.1%	73.4%
3	17.5%	49.9%	23.2%	71.6%
4	15.2%	45.9%	23.0%	70.8%
24	12.3%	43.0%	20.8%	68.0%

Example 9:

Residual efficacy testing of water resistant antimicrobial coating on ceramic tile: Repeat challenges (PSA 15442)

Antimicrobial compositions were prepared as described in Examples 7 and 8. 8μL product/cm^2 substrate was dispensed onto the glazed ceramic tile samples and distributed with a sterile glass spreader and allowed to dry. The samples were then rinsed in a circulating water bath at 50°C with hard water (50 ppm $CaCO_3$) for 1 hour.

To challenge, 250μL of 1x10^6 cfu/mL of *Pseudomonas aeruginosa* (ATCC # 15442) in PBS was dispensed as drop inoculum onto the samples and incubated for 20 hours at room temperature. The rinse, challenge and incubation cycles were repeated 10 times.

Quantitation was performed by the standard serial dilution / spread plate method. Results are shown in Figure 3.

Example 10:

Residual efficacy testing of water resistant antimicrobial coating on stainless steel: Repeat challenges (PSA 15442)

Antimicrobial compositions were prepared as described in Examples 7 and 8 and applied to stainless steel samples having a surface area of 6.45 cm^2 as described above. The samples were contacted with hard water and challenged with *P. aeruginosa* as described above.

Results showing the residual efficacy of the antimicrobial coatings after challenge are shown below in Table III.

	Log_{10} Reduction (Relative to Control)			
Film	1 hr	2 hrs	4 hrs	8 hrs
PHMB	-0.3	1.0	0.4	0.0
PHMB/SDS	1.8	3.2	3.9	4.6
PHMB/SDS/Ag	1.5	3.0	5.5	4.7
M-PHMB	-0.6	-0.9	-0.2	-0.2
M-PHMB/SDS	1.5	3.6	2.2	2.5
M-PHMB/SDS/Ag	3.6	3.9	4.9	4.0

Claims:

1. An amphiphilic antimicrobial film-forming composition comprising:

   a) an antimicrobial polymer comprising a plurality of cationic subunits,

   b) an anionic compound comprising an anionic moiety and a hydrophobic moiety, and

   c) a liquid carrier,

   wherein the ratio of the number of anionic moieties to the number of cationic subunits in the composition is between 0.05 and 0.95.

2. The composition of claim 1, wherein the composition deposits an antimicrobial film when contacted by a hydrophobic substrate.

3. The composition of claim 1, wherein the composition deposits an antimicrobial film when contacted by a hydrophilic substrate.

4. The composition of claim 1, wherein the composition forms a substantially water-insoluble film.

5. The composition of claim 1, wherein the composition forms a film from which the polymer does not leach into water at biocidal levels.

6. The composition of claim 1, wherein the composition forms a film that provides antimicrobial activity that persists after contact with water.

7. The composition of claim 1, wherein the antimicrobial polymer is a biguanide polymer.

8. The composition of claim 7, wherein the biguanide polymer is poly(hexamethylenebiguanide) or a derivative thereof.

9. The composition of claim 8, wherein the derivative is an adduct formed by reaction of poly(hexamethylenebiguanide) with a reactant comprising a hydrophobic moiety.

10. The composition of claim 9, wherein the reactant is methylene-bis-N,N-diglycidylaniline.

11. The composition of claim 1, wherein the anionic moiety is selected from the group consisting of a sulfate, a sulfonate, a phosphate, a phosphonate, a borate, and a carboxylate.

12. The composition of claim 11, wherein the anionic moiety is a sulfate.

13. The composition of claim 11, wherein the anionic moiety is a sulfonate.

14. The composition of claim 11, wherein the anionic moiety is a carboxylate.

15. The composition of claim 11, wherein the ratio of the number of anionic moieties to the number of cationic subunits in the composition is between 0.2 and 0.8.

16. The composition of claim 1, wherein the anionic compound comprises a hydrophobic moiety comprising an aliphatic substituent having at least twelve carbon atoms.

17. The composition of claim 16, wherein the anionic compound is dodecyl sulfate.

18. The composition of claim 16, wherein the anionic compound is stearate.

19. The composition of claim 16, wherein the anionic compound comprises an aliphatically substituted aryl moiety.

20. The composition of claim 19, wherein the aliphatically substituted aryl moiety is dodecylbenzene.

21. The composition of claim 1, wherein the liquid carrier is selected from the group consisting of water, a polar organic solvent, mixtures of polar organic solvents, and mixtures of water with one or more polar organic solvents.

22. The composition of claim 21, wherein the liquid carrier is selected from the group consisting of a C_1 to C_4 alkanol, a C_1 to C_4 nitrile, a ketone comprising from three to eight carbon atoms, dimethylsulfoxide and N,N-dimethylformamide.

23. The composition of claim 22, wherein the liquid carrier is ethyl alcohol.

24. The composition of claim 21, wherein the liquid carrier comprises a mixture of water and a polar organic solvent, wherein the water is present at a concentration of at least 20% (w/v).

25. The composition of claim 1, wherein the composition does not include a metal.

26. The composition of claim 1, wherein the composition further comprises a transition metal.

27. The composition of claim 26, wherein the composition deposits an antimicrobial film on a contacting substrate, and wherein the film comprises the antimicrobial polymer, the anionic compound, and the metal.

28. The composition of claim 26, wherein the composition deposits an antimicrobial film on a contacting substrate, and wherein the presence of the metal in the composition reduces the water-solubility of the film.

29. The composition of claim 26, wherein the metal is a biocide.

30. The composition of claim 26, wherein the metal is silver.

31. A method of preparing a film-forming composition, the method comprising the steps of:

a) providing a solution comprising a polar organic solvent and an antimicrobial polymer comprising a plurality of cationic subunits, and

b) adding to the solution an anionic compound comprising a anionic moiety and a hydrophobic moiety,

wherein the final ratio of the number of anionic moieties to the number of cationic subunits in the composition is between 0.05 and 0.95.

32. The method of claim 31, wherein step a) comprises preparing an aqueous solution of the antimicrobial polymer and mixing the aqueous solution with a polar organic solvent.

33. The method of claim 31, wherein the anionic moiety is present in an aqueous solution prior to its addition to the solution comprising the polar organic solvent.

34. A method of immobilizing an antimicrobial polymer on a substrate, the method comprising the steps of:

a) preparing a solution comprising i) an antimicrobial polymer comprising a plurality of cationic subunits, ii) an anionic compound comprising a anionic moiety and a hydrophobic moiety and iii) a polar organic solvent, such that the concentration of the polar organic solvent is always at least 0.5% (w/v) and the molar ratio of the number of anionic moieties to the number of cationic subunits in the solution is never more than 5, and such that the final ratio of the number of anionic moieties to the number of cationic subunits in the solution is between 0.05 and 0.95, and

b) contacting a substrate with the solution, such that the antimicrobial polymer and the anionic compound form a water-resistant film on the substrate.

35. An antimicrobial film comprising

a) an antimicrobial polymer comprising a plurality of cationic subunits, and

b) an anionic compound comprising a anionic moiety and a hydrophobic moiety,

wherein the molar ratio of anionic moieties to cationic subunits in the film is between 0.05 and 0.95, and

wherein the film is resistant to extraction with water, and provides a persistent antimicrobial activity against contacting microorganisms.

36. The antimicrobial film of claim 35, wherein the antimicrobial polymer does not elute from the film into contacting water at biocidal levels.

37. A method of depositing an antimicrobial film on a substrate, the method comprising the step of contacting the substrate with the composition of claim 1.

38. A substrate contacted with the composition of claim 1.

39. A substrate coated with the film of claim 35.

40. A film created by the process of claim 37.

41. A composition created by the process of claim 31.

42. An amphiphilic antimicrobial film-forming composition comprising:

a) an antimicrobial polymer comprising a plurality of cationic subunits,

b) an anionic compound comprising a hydrophobic moiety and a anionic moiety selected from the group consisting of phosphate and phosphonate, and

c) a liquid carrier,

wherein the ratio of the number of anionic moieties to the number of cationic subunits in the composition is greater than two.

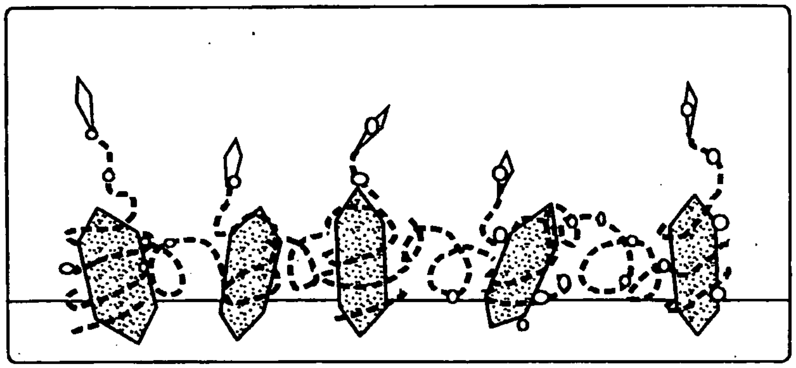


FIG. 1A

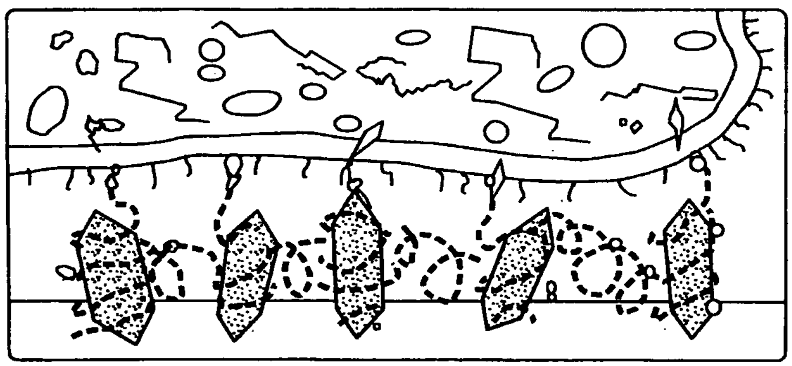


FIG. 1B

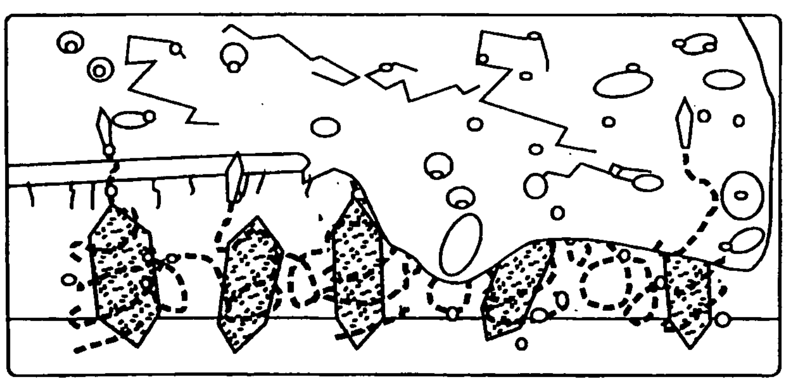
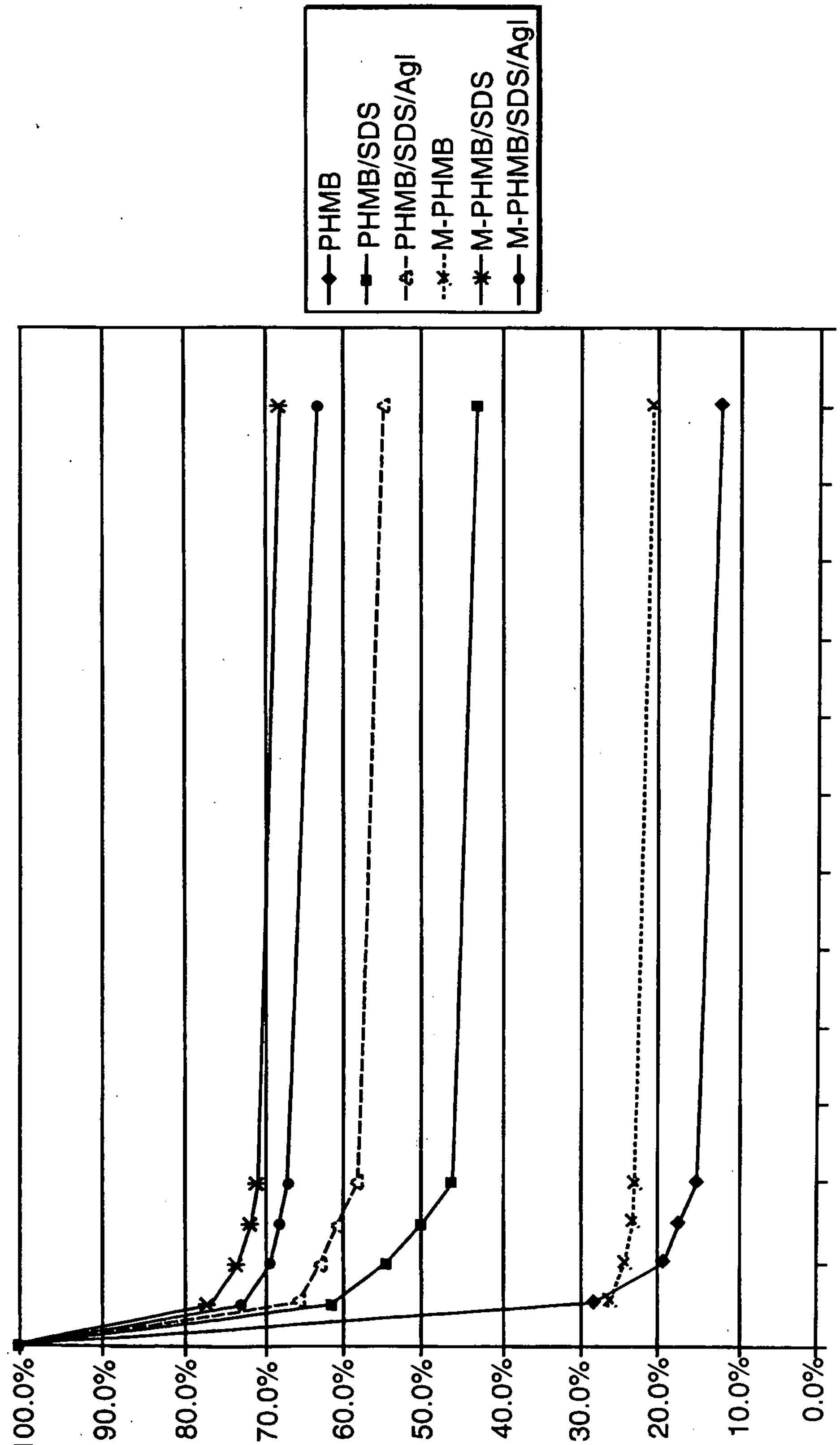


FIG. 1C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M-PHMB
M-PHMB/SDS
M-PHMB/SDS/AgI
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80.0%
70.0%
60.0%
50.0%
40.0%
30.0%
20.0%
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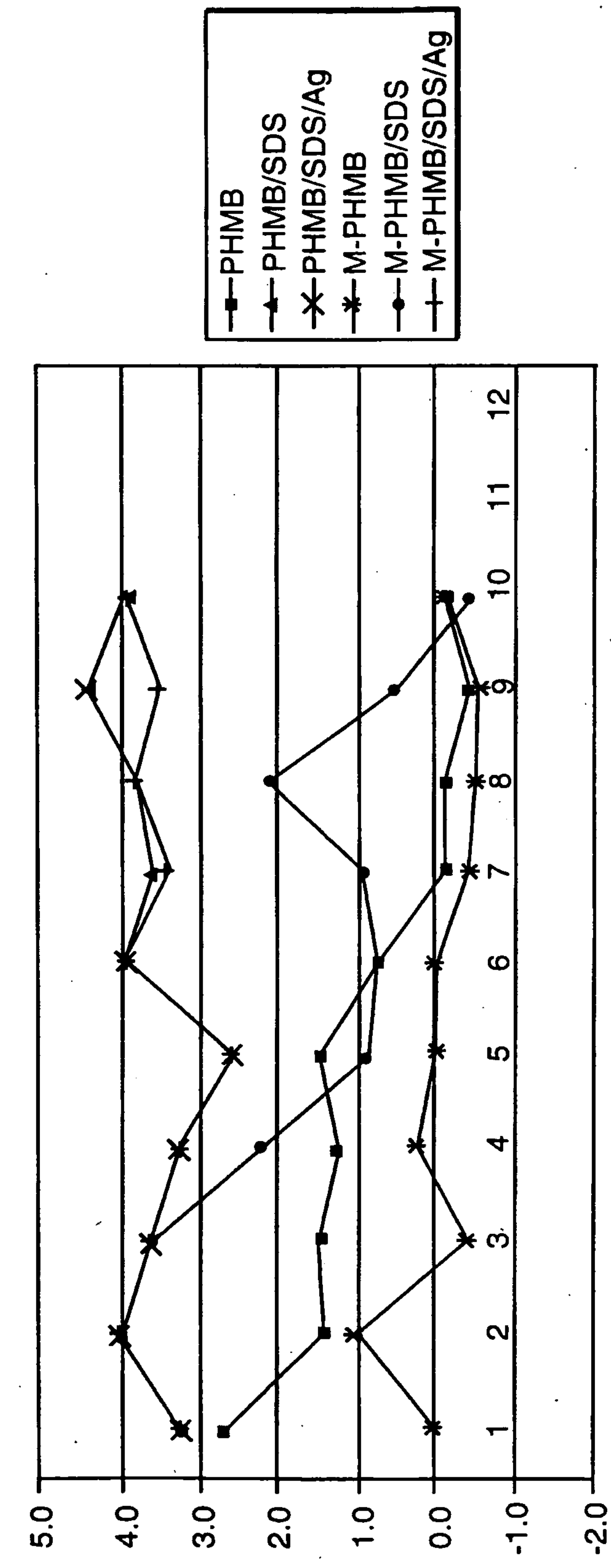
FIG. 2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PHMB/SDS/Ag
M-PHMB
M-PHMB/SDS
M-PHMB/SDS/Ag
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3.0
2.0
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0.0
-1.0
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FIG. 3

INTERNATIONAL SEARCH REPORT

Interr ıal Application No
PCT/US 00/06053

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 A01N47/44 A01N25/34 A01N25/24 A01N33/12

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 A01N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 99 40791 A (SURFACINE DEV COMPANY LLC) 19 August 1999 (1999-08-19) claim 23; examples 2B,2C,2E,3-11	1-42
E	WO 00 15036 A (SURFACINE DEV COMPANY LLC) 23 March 2000 (2000-03-23) examples 1,2,8	1-42
A	WO 98 18330 A (SURFACINE R CONSUMER PRODUCTS ;BIOPOLYMERIX INC (US)) 7 May 1998 (1998-05-07) the whole document	1-42
A	EP 0 460 385 A (FULLER H B LICENSING FINANC) 11 December 1991 (1991-12-11) the whole document	1-42

☐ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

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Date of the actual completion of the international search	Date of mailing of the international search report
11 July 2000	18/07/2000

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INTERNATIONAL SEARCH REPORT

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